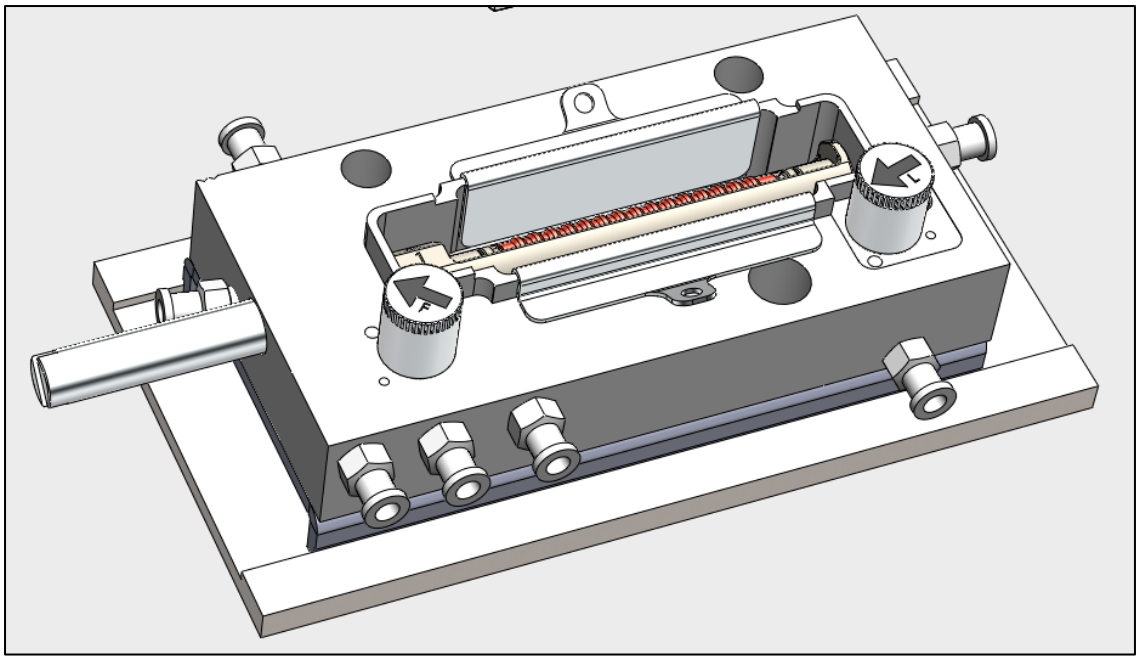
MiniBreath Bioreactor™

Operator's Manual



HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

84 October Hill Drive, Holliston, MA 01746 USA
www.HARTregen.com 774.233.7300[tele]

Contents

Contents	2
Overview	3
Symbols	4
Warning & Caution Statements	5
General Safety Requirements	5
Chapter 1 - Introduction	
1.1 Equipment Components	7
1.2 Required Supplies & Equipment	10
1.3 Differences between Rev 3 & Rev 4	11
Chapter 2 - Operating Instructions	
Sterilization	13
Surface Disinfection	15
Post-Sterilization Assembly	16
Fluid Connections & Stimulation	17
Cell Seeding	18
Chapter 3 - Care & Maintenance	
Cross-Infection Prevention / Universal Precautions	20
Cleaning	20
Specifications	20
Troubleshooting	21
Frequently Asked Questions	22
Parts List	23
Update Log	24

HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

Harvard Apparatus Regenerative Technology (HART) partners with leading global scientists to provide specialized solutions.

The company is uniquely positioned to develop advanced instrumentation to accelerate regenerative medicine, tissue engineering and cell therapy experimentation. From the beginning, we worked closely with leading global researchers to produce products with the highest levels of performance, quality and support necessary for the new challenges of your life science research.

We look forward to working with you to develop new tools to assist you in solving the new challenges of regenerative medicine from the lab bench to the patient. There are thousands of publications, in regenerative medicine to stem cell research, utilizing HART products, but we are now introducing some newly developed products: one for regenerative organ generation and one for small volume cell delivery into organs. These products will serve the researcher and the physicians to accelerate the research and utilization of that research in patients.

Disclaimer:

Use of the MiniBreath™ Bioreactor should be conducted by a trained and manufacturer qualified representative. Harvard Apparatus Regenerative Technology (HART) does not warrant unauthorized use of this product; HART does not warrant that the operation of this product will be uninterrupted or error-free and makes no claim of warranty or condition.

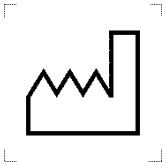
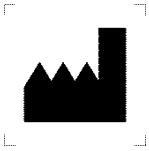
HART reserves the right to change the instructions for use and any related products at any time without any prior notice and is not liable for any damages arising out of any change and/or alteration of the contents or product.

This product is for RESEARCH USE ONLY.

Copyright © 2013, Harvard Apparatus Regenerative Technology. All rights reserved.
MiniBreath™ is a trademark of Harvard Apparatus Regenerative Technology.

Harvard Apparatus Regenerative Technology owns the intellectual property rights to the MiniBreath Bioreactor. This material may not be reproduced, displayed, modified, or distributed without the expressed prior written permission of the copyright holder.

U.S., international, and foreign patent applications are pending.

Symbol	Definition
	Date of Manufacture
	Serial Number
	Catalogue Number
	Warning: This action will have a direct impact on the patient
	Caution: This action will have an impact on the product or operator
	Manufacturer
	This device complies with Directive 2006/95/EC relating to electrical equipment designed for use within certain voltage limits; this device also complies with Directive 2004/108/EC relating to electromagnetic compatibility.

Warning and Caution Statements

The use of a **WARNING** statement in this User Manual alerts you to a potential safety hazard. Failure to observe a warning may result in a serious injury to the user.

The use of a **CAUTION** statement in this User Manual alerts you to where special care is necessary for the safe and effective use of the product. Failure to observe a caution may result in minor injury to the user or damage to the product or other property.

Intended Use

The MiniBreath Bioreactor is a vessel used to support a hollow organ scaffold for the purpose of cell-seeding; it is intended for **research use only.**

WARNING:

Use of this device in non-research settings must be conducted under local Regulatory requirements; consult your local Regulatory Authority.

The following conditions must be met prior to using the Bioreactor:

General Safety Requirements

WARNING:

The Bioreactor should only be used by qualified personnel who have been trained by the manufacturer or other authorized representative. Unauthorized use of this device is not recommended.

WARNING:

To prevent contamination, aseptic procedures must be followed and personal protective equipment must be worn at all times when handling and using the Bioreactor.

WARNING:

Wherever blood products are used, Universal Precautions must be followed.

WARNING:

DO NOT SUPPLY EXPLOSIVE GASES TO THE BIOREACTOR

Facility Requirements

Assure that the facility is able to provide a clean, safe, and suitable area for aseptic cell processing. It is recommend that all manipulations of the unit once sterile are performed in a biological safety cabinet (laminar flow hood).

WARNING:

Failure to provide a means to conduct aseptic cell processing may result in harmful contamination.

Chapter 1: Introduction

The **MiniBreath Bioreactor** is a rotating, double chamber bioreactor designed for cell seeding and culturing both surfaces of a tubular matrix and includes rotational movement of the scaffold around its longitudinal axis.

A polymeric chamber houses the biological materials throughout the culture period. Cylindrical scaffold holders are constructed with working end of different diameters—to house matrices of diverse dimensions—and a central portion of smaller diameter to expose the luminal surface of the matrix for cell seeding and culturing.

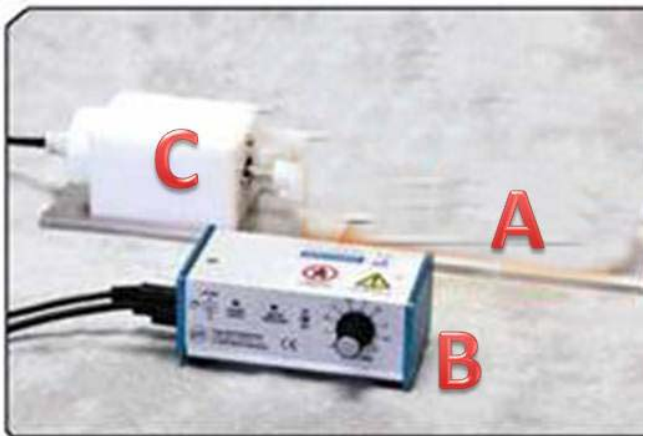
A co-axial conduit links the inner chamber to the external environment through the chamber wall. This provides access to seed and feed the luminal surface of the construct. Secondary elements moving with the scaffold holder induce continuous mixing of the culture medium to increase oxygenation and mass transport.

1.1 Equipment Components

(A) Drive Motor Base Plate: Plate that aligns the reservoir with the drive motor.

(B) Control Unit: Independent controller of rotational speed. The bioreactor may also be controlled using an ORCA Controller.

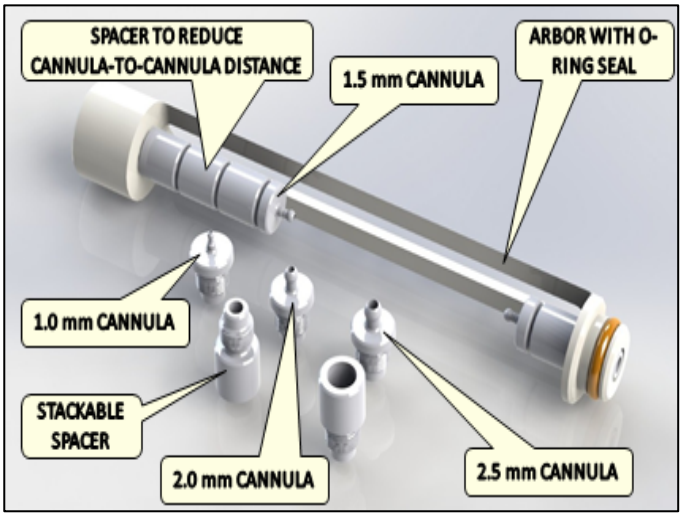
(C) Motor Drive: Drive motor responsible for rotation of the Arbor and organ.



Arbor Assembly - General Overview

The size of the organ required will dictate the number and size of inserts required. The organ size can vary from a 1.0—2.5mm ID, 9.4mm OD and a length of 7.7-65mm.

Spacers are placed on either end of the arbor assembly. A minimum of two spacers are used with each Arbor (one for each end) and the cannula are inserted into the spacers. A maximum of 8 of the 5mm spacers can be used. Spacers are available in 5mm, 10 and 17mm.

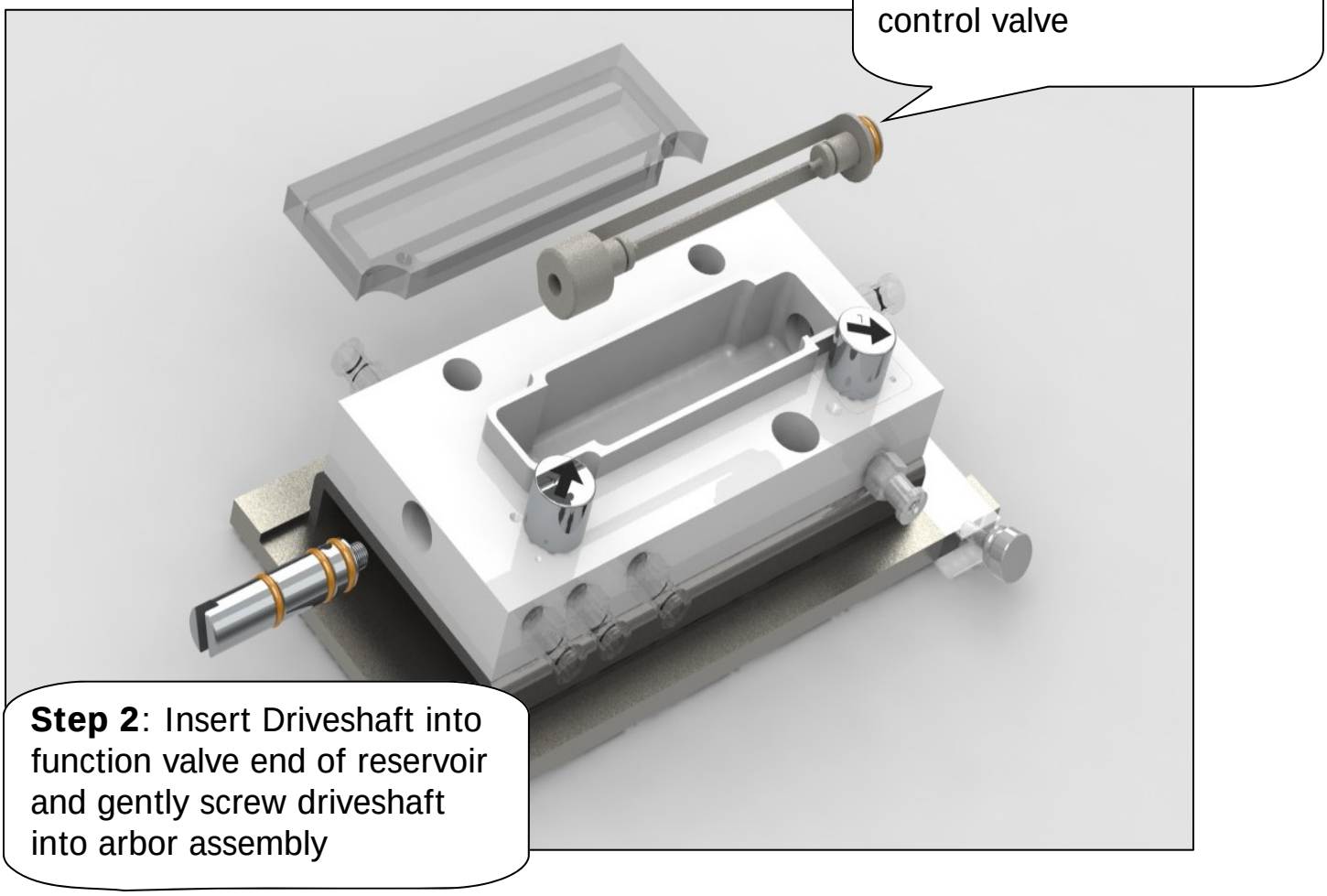


The cannula pieces are dictated depending on the inner diameter of the organ. Typical choices are: 1.0mm, 2.0mm, 2.5mm, and 3.0mm.

Step #1: Prepare the Arbor assembly by selecting the correct number of spacers and the correct cannula and mounting them onto the Arbor

Step #2: Attach the organ if it is to be decellularized by suturing it into place.

Note: During recellularization the organ should be mounted inside a laminar flow hood by an operator trained in aseptic technique and only after the bioreactor has been sterilized.

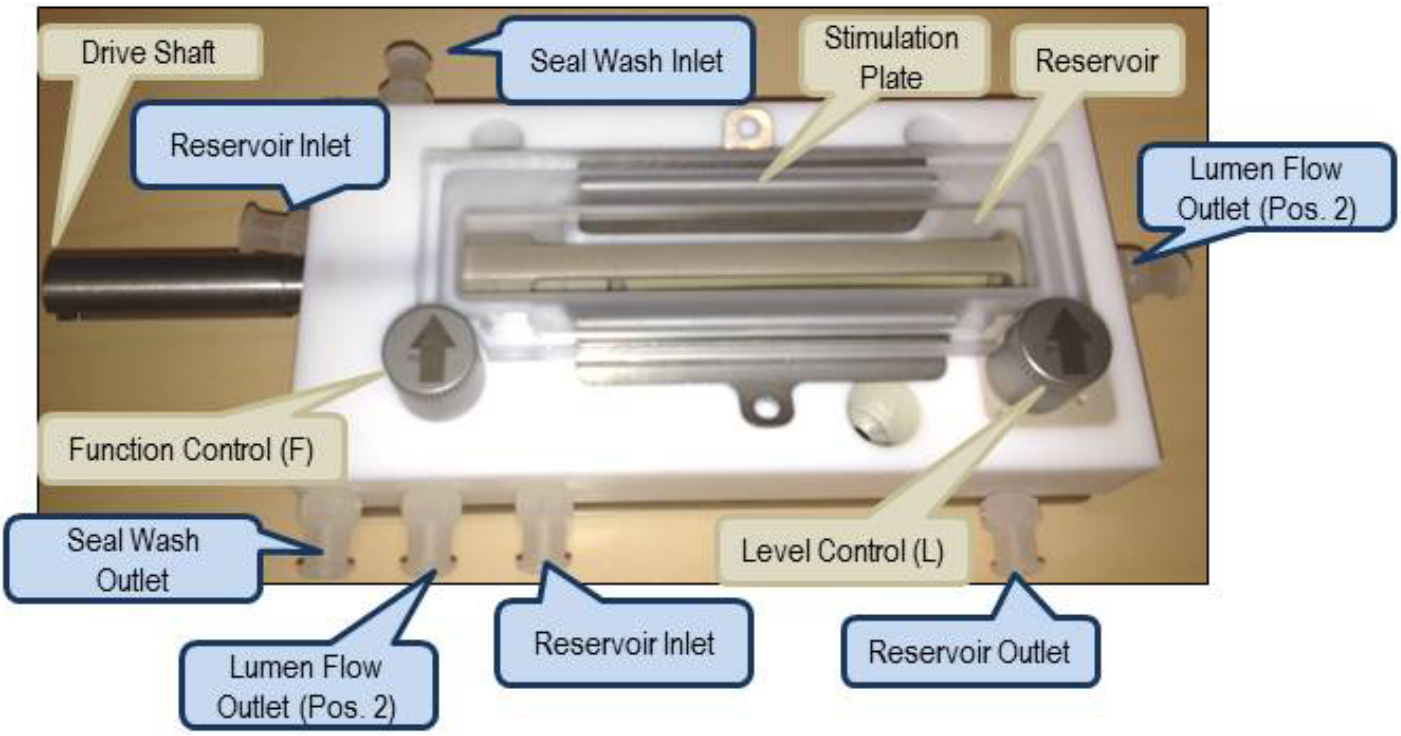

Step 1: Insert O-Ring end of arbor assembly into hole in reservoir near level control valve
Step 2: Insert Driveshaft into function valve end of reservoir and gently screw driveshaft into arbor assembly

Driveshaft:
Connecting shaft that links the motor to the arbor allowing for rotational control

Reservoir:
Cavity allowing the arbor and organ to sit inside a selected volume of reagents or medium

Function Control:
Control knob that allows selection of multiple flow paths

Level Control:
Knob for selecting the level of medium in the reservoir



Flow Path Selection

Using the selector knob on the lower left side (marked “F”) select the position that indicates the flow path required:

Position 1: Lumen flow blocked; recirculates the medium / reagent back into the reservoir.
Position 2: Lumen flow to lumen flow outlet (middle connector on bottom left)
Position 3: Lumen flow is re-circulated into the reservoir
Position 4: Low volume level in reservoir
Position 5: High volume level in reservoir

1.2 Required Supplies & Equipment

MiniBreath Bioreactor Components

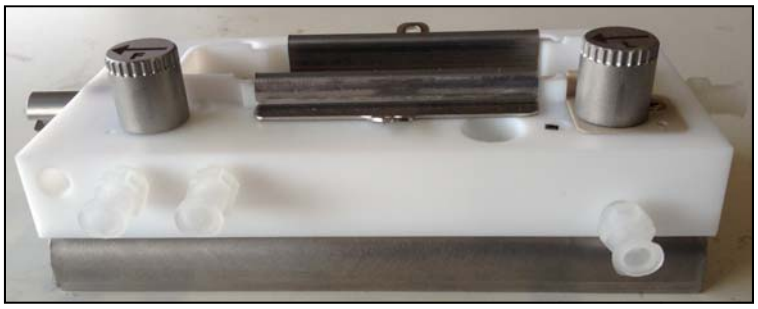
Equipment	Part Number
LV Bioreactor	30190
Motor Unit	30170
Control Unit	30280
Power Adapter	
Drive Shaft Assembly	30203
Arbor Assembly	30192
Spacers (two mounted on Arbor, others if ordered)	31056
1.0 mL Cannula (if ordered)	31051
1.5 mL Cannula (mounted on Arbor)	31052
2.0 mL Cannula (if ordered)	31053
2.5 mL Cannula (if ordered)	31054
3.0 mL Cannula (if ordered)	31055

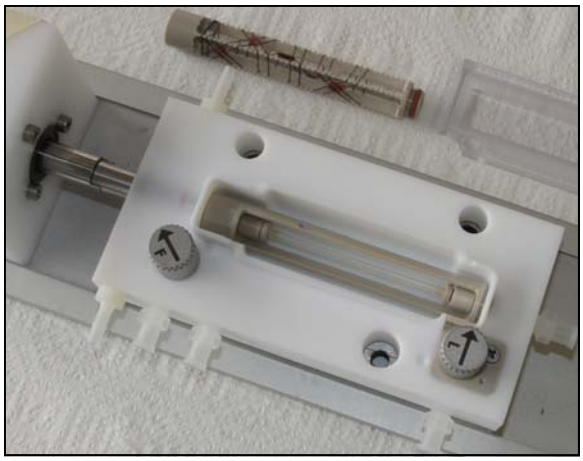
Non-Bioreactor Components

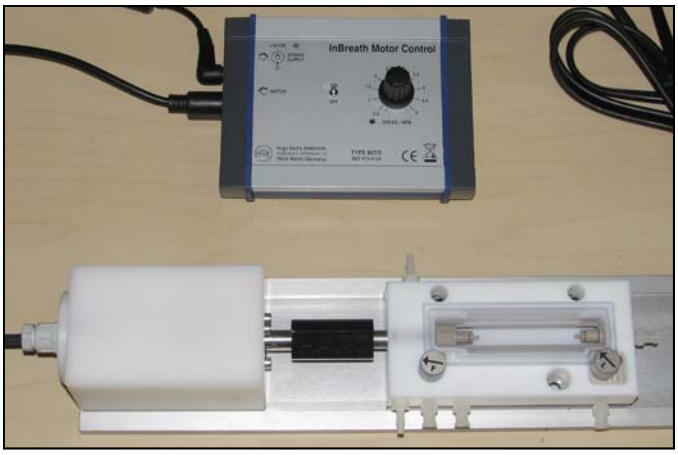
Sterilizing Strips
Sterilizing Bags (250mm)
Sterilizing Bags (350mm)
Ethanol 70% (1L)
Surgical suture thread with needle (size 4/0 or 3/0)
Sterile serological pipette
Sterile drape
Sterile bottle (1L)
PBS buffer, clinical grade (750ml)
Culture medium (280ml)
Inoculum (40ml)
Male Luer integral lock ring plug (sterile)
Laminar Flow Hood
Adjustable Pipette
CO_2 Incubator
Repipette

1.3 Differences between Revision 3 & Revision 4

Rev 3

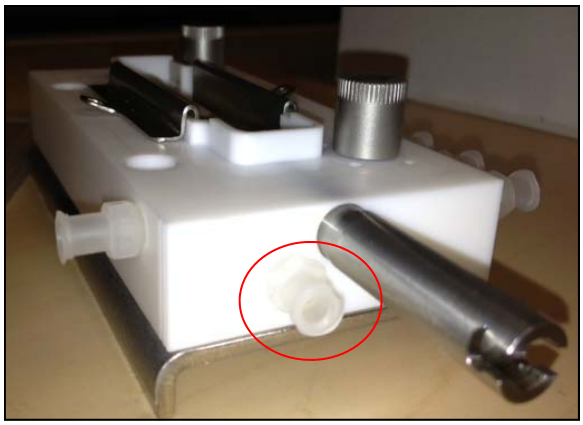


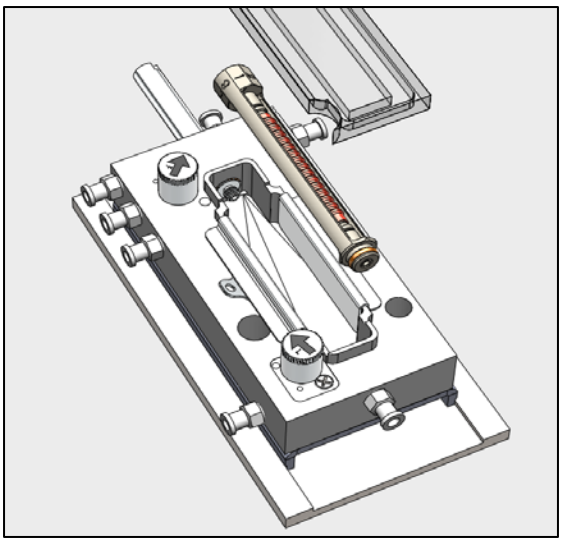




Rev 4

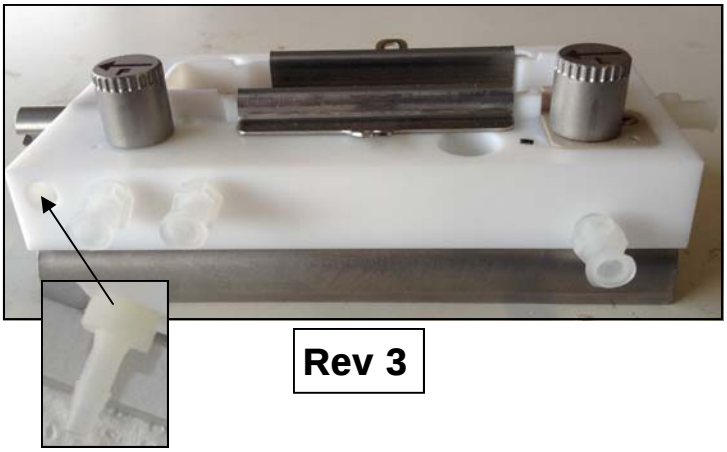
This new port (see image, below left) is used as a cell sweeping inlet port. The bottom of the reservoir is now designed at an angle; there is a downward slope from the motor side of the bioreactor to the outlet side. Recirculating reservoir media into this top port will effectively sweep accumulated cells down the incline towards the outlet passage.





The arbor is now labeled with the numbers 1, 2, 3, & 4 for easy recognition of the speed of rotation.

The ports are now all standardized so that each are the same size; they are interchangeable. On the Rev 3, some of the ports had a smaller diameter.



Rev 3

Rev 4

Swabble ports are available for ports not being used.

Chapter 2: Operating Instructions

Prior to Starting:

Assure that all the requirements identified in Chapter 2 have been successfully met.

Personnel using the MiniBreath Bioreactor should read through this procedure in its entirety prior to using the device.

Personnel should train on all processes and equipment prior to operating the Bioreactor.

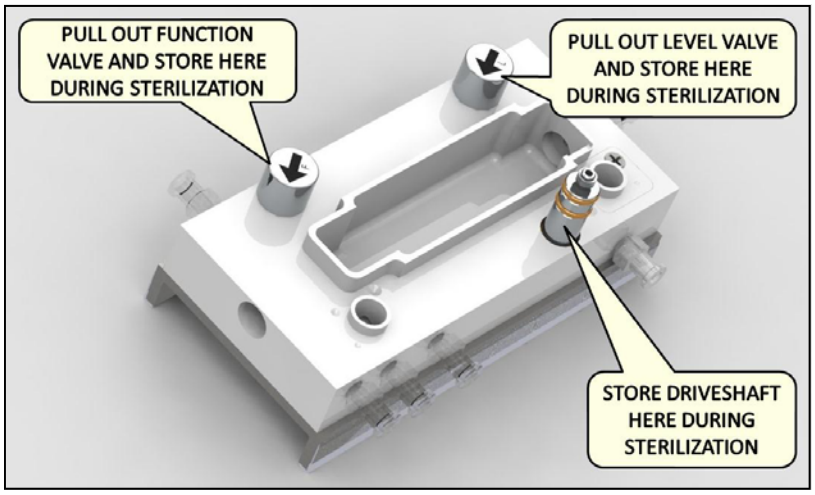
WARNING:

Failure to follow aseptic techniques and failure to train on all processes and procedures prior to using the In-Breath Bioreactor may result in critical delays, contamination, and other harmful events

Sterilization

Bioreactor Equipment for Sterilization

Sterile Pouch	Equipment
Bag #1	Culture vessel
Bag #2	Arbor Assembly
	Drive Motor
	Base Plate
	Control Unit
	Power Supply


PULL OUT FUNCTION VALVE AND STORE HERE DURING STERILIZATION
PULL OUT LEVEL VALVE AND STORE HERE DURING STERILIZATION
STORE DRIVESHAFT HERE DURING STERILIZATION

The bioreactor was developed in order to allow for multiple sterilizations via standard laboratory procedures, including autoclavation, ethylene oxide sterilization and plasma sterilization. During sterilization processes, sealing surfaces such as Teflon stainless steel or Teflon o-ring should be disassembled to prevent permanent distortion.

Sterilization steps:

1. Remove function valve “F” and store in the upper left storage port
2. Remove function valve “L” and store in the upper right storage port
3. Remove Drive Shaft and store in center right storage port
4. Remove arbor assembly from chamber
5. Place the bioreactor into the appropriate sterilization bag or container.

After sterilization, place the unit into a laminar flow hood for final assembly to minimize contamination.

Before autoclaving, foil or autoclave wrap the following:

1. Reservoirs and connectors

2. Tubing ends

3. Luer fittings of all bioreactor connections (do not unscrew).

Then, remove valves and drive shaft from the operating ports and place them into the storage ports.

Surface Disinfection

Equipment
Drive Motor & Base Plate
Control unit
Power Supply

1. Disassemble the bioreactor into its components and place the parts according Table #02 into individual sterilization packages.
2. Disassemble the Arbor (organ holder) prior to sterilization. The driveshaft must be unthreaded to allow the arbor to be removed. Place the arbor into the sterilization pouch.
3. Follow the packaging instruction for sterilization. The packaging must be suitable for weight and geometry of the bioreactor parts and for the sterilization process.
4. Ensure that the each pouch has a chemical indicator strip for sterilization.
5. Label the packages according table 02, column “Equipment”
6. Perform sterilization process on the sterile packaged bioreactor equipment.
7. Confirm all the sterilization pouches are returned and there are no visual issues. (use table 02 for a list).
8. Inspect each pouch for integrity (no bubbles, no wrinkles) and review the sterilization indicator.
9. Transfer the sterilization pouches to the clean room facility.

Preparing Non-Sterilizable Equipment

1. Wipe the equipment listed in table 03 with 70% medical grade Ethanol. Take care not to wet the electrical connections, switches, knobs, etc.
2. Transfer the disinfected equipment to the clean room facility.

CAUTION:

In the case where several bioreactors are being used simultaneously, a unique identifier should be placed on each bioreactor component to avoid parts being exchanged. Colored stickers have typically been used on each pouch. Similar precautions should be taken with components post sterilization.

WARNING:

Failure to follow the defined sterilization procedure may damage the bioreactor and make it unsuitable for use

Assembly Preparation

The bioreactor parts are delivered in sterile pouches. Unpacking and bioreactor assembly should be performed in a clean room environment provided by a laminar airflow cabinet.

CAUTION:

To prevent contamination, aseptic procedures must be followed and personal protective equipment must be worn at all times when handling and using the Bioreactor.

CAUTION:

Failure to strictly follow aseptic techniques may result in critical delays, contamination, and other harmful events.

Post-Sterilization Assembly

NOTE:

Two operators are recommended for unpacking and assembly.

Sterile Bioreactor Equipment

Bioreactor
Arbor & organ sterilized in sterile pouch
Drive shaft
Reservoir Cover
Transport Cover

Other Materials

Surgical suture thread with needle
Sterile serological pipette
Sterile bottle (1000 ml)
PBS buffer
Culture medium
Inoculum

Disinfected Bioreactor Equipment

Drive unit
Control unit
Power Supply

After sterilization, prepare the arbor assembly and organ in a ***laminar flow cabinet*** and then install the arbor into the Bioreactor reservoir. Cover the working surface for bioreactor assembly with a sterile cloth.

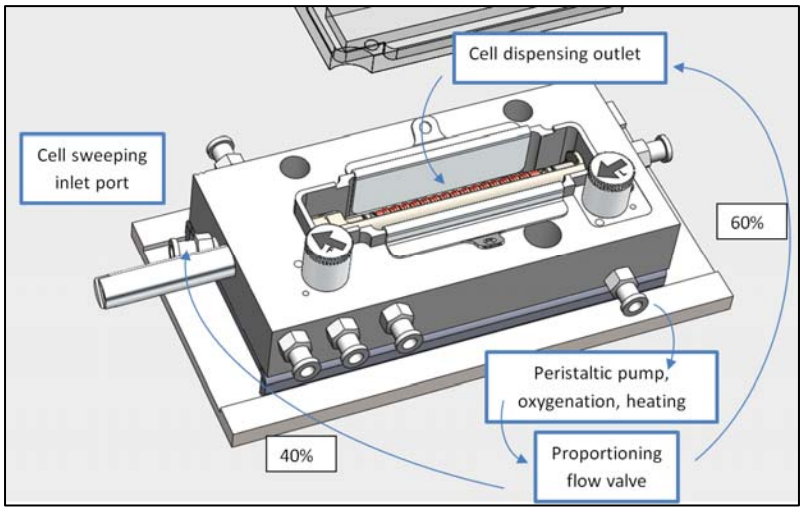
Assembly steps:

1. Insert the O-Ring end of the Arbor assembly into the hole in the reservoir near the level control valve. Lower the free end of the Arbor into the reservoir cavity.
2. Insert the drive shaft into the function valve end of the reservoir and gently screw the drive shaft into the arbor assembly. Place you finger lightly on the top of the wide end of the arbor to prevent it from turning.
3. Place the cover on the Bioreactor. The cover is a loose fit allowing gas permeability while in an incubator.
4. Final assembly is recommended to be carried out in a laminar flow hood to minimize contamination. Normal aseptic technique is recommended in wiping down the flow path that has been sterilized when bringing it into the hood.

Note: If the Bioreactor was sterilized in one location and must be transported to another, the use of a transport box is recommended. If the organ is to be transported from another building, it is recommend that the transport box with the Bioreactor inside be placed in a thermal maintenance container to minimize heat loss.

Fluid Connections

Suggestion for recirculation of media to organ with maximum cell concentration.



The Seal Wash Inlet–Outlet is designed to take a non-salt solution and flush it through a separate space between the two outside O-rings on the driveshaft. This will prevent any buildup of evaporated salt crystals from forming on the turning shaft during long periods of use. An intravenous drip bag filled with water set to between 0.1 and 0.5mL per minute can be used to continuously flush the seal wash path.

Lumen Flow Inlet should be connected to a peristaltic pump that will deliver the medium through the lumen. Reservoir Inlet should be connected to a peristaltic pump that will deliver the medium to the exterior of the organ into the reservoir.

Note: Depending on the selection of the ”F” selector knob, the outlet of the lumen may change from the lumen flow outlet to the reservoir.

Stimulation

Stimulation plates may be added to the bioreactor. Round eye connection tabs are present to connect to a stimulator. See the individual stimulator manual for connection instructions.

Cell Seeding Preparation

1) Wipe a motor unit and its cable with 70% Ethanol.
2) Place the motor unit inside the BSC laminar flow cabinet.
3) Connect motor and control unit.
4) Place the culture vessel onto the drive unit.

The following is a typical cell seeding preparation. Actual procedure should be determined by individual laboratory protocols.

First PBS wash:

1) Add PBS-Buffer with a pipette to the culture vessel.
2) Place the culture lid onto the bioreactor vessel.
3) Rotate the scaffold for 20 min at 5 rpm.
4) Stop the bioreactor agitation.
5) Remove the washing buffer with a pipette completely from the culture vessel.

Second PBS wash:

1) Add PBS-Buffer with a pipette to the culture vessel.
2) Place the culture lid onto the bioreactor vessel.
3) Rotate the scaffold for 20 min at 5 rpm.
4) Stop the bioreactor agitation.
5) Remove the washing buffer with a pipette completely from the culture vessel.

Third PBS wash:

1) Add PBS-Buffer with a pipette to the culture vessel.
2) Place the culture lid onto the bioreactor vessel.
3) Rotate the scaffold for 20 min at 5 rpm.
4) Stop the bioreactor agitation.
5) Remove the washing buffer with a pipette completely from the culture vessel.

Scaffold conditioning:

1) Add culture medium with a pipette to the culture vessel.
2) Place the culture lid onto the bioreactor vessel.
3) Rotate the scaffold for 120 min at 2 rpm.
4) Stop the bioreactor agitation.
5) Remove the culture medium with a pipette completely from the culture vessel.

Selection of Fluid Paths:

Select position for “F” function control switch. Fill medium reservoirs.

Media fill:

Add culture medium with a pipette to the culture vessel.

Cell Seeding

1) Add inoculum onto the graft surface. Rotate the scaffold for 10 min at 2 rpm. Inspect if the scaffold moves evenly together with the organ holder. Place the culture lid onto the bioreactor vessel. Stop the bioreactor agitation.

WARNING:

The scaffold must rotate during the cell seeding process.

2) Place the motor unit into the cell culture incubator. Wipe an incubator feed through cable with 70% Ethanol. Connect the cable to the motor and control unit.
3) Place the seeded culture vessel on the drive unit inside the cell culture incubator. Start bioreactor rotation at 2 rpm at 37°C and 5 % carbon dioxide atmosphere. Monitor the scaffolds to assure it turns evenly.
4) The bioreactor is ready for the incubation process. Follow the working instruction for bioreactor incubation. Inspect the scaffold rotation in regular intervals.

Chapter 4: Care & Maintenance

Cross-Infection Prevention, Biohazardous Waste, and Product Disposal

Cross-Infection Prevention/Universal Precautions

All blood products or products potentially contaminated by blood or other body/animal fluids should be treated as potentially infectious materials. Personal protective equipment should be worn at all times when using the MiniBreath Bioreactor to protect personnel from becoming contaminated as well as to help prevent cross-infection and cross-contamination.

Bench tops, equipment, and other potentially contaminated surfaces should be cleaned and disinfected according to the manufacturers' and/or the facility's procedures. Any article used to clean potentially contaminated surfaces should be disposed of as Biohazardous Waste.

CAUTION:

Failure to use the manufacturers' cleaning and disinfecting procedure could result in damage to the surface or equipment.

Biohazardous Waste

Dispose of biohazardous waste according to local Regulatory requirements.

Cleaning

Flush all fluid paths with deionized water and either follow with or wipe with 70% IPA.

These materials of construction will withstand virtually all biological reagents and cold sterilization agents.

Stainless steel parts may be sonicated.

Specifications

Size of Organ	1.0—2.5 mm ID, up to 65 mm in length is standard
Rotation Speed	0 - 5 rpm
Diagnostics	Positional Monitoring
Materials	Autoclavable Teflon, PEEK and Stainless Steel
Power	100-240 VAC, 50/60 Hz

Troubleshooting

Arbor not turning:

Check to see if the reservoir is in place and the drive shaft is connected to the motor. The retaining clamp is tightened during installation to maintain the connection.

Check to see if the medium—blood being used has fouled the arbor. This can occur in high fibrinogen solutions.

Excess Medium / Reagent build up in the reservoir:

Check for clogging or fouling of the reservoir outlet line or port especially during a decellularization procedure.

Medium level in the IC or EC flow path has gone dry:

Confirm that there is sufficient medium in the inlet reservoirs and that the inlet tubing inside the reservoirs is below the medium level.

Leaks:

DO NOT USE BLEACH WHEN CLEANING THE BIOREACTOR AS IT HAS BEEN SHOWN TO CAUSE FITTINGS TO DEFORM.

Also, be sure to check O-Rings and replace as necessary.

Frequently Asked Questions

Is the retainer clamp missing from my unit?

The retainer clamp design did not fit as well as it was supposed to, and since it did not seem to be necessary, we have not been shipping it with the product. A new clamp is in the process of being developed to make sure the drive mechanism does not slip and disengage itself.

How does the "seal wash inlet" and outlet work?

This passage is designed to take a non-salt solution and flush it through a separate space between the two outside o-rings on the driveshaft. This will prevent any buildup of evaporated salt crystals from forming on the turning shaft during long periods of use. As an example, an intravenous drip bag with water set to between 0.1mL and 0.5mL per minute to continuously flush the seal wash path could be used.

Should I sterilize using plasma sterilization or does autoclavation work?

The materials of construction are Teflon reservoir, stainless steel baseplate, driveshaft, and valves, silicone rubber o-rings, Kynar luer fittings, polycarbonate cover, and PEEK arbor components. All of these materials will withstand steam sterilization.

One CAUTION, however.... **Please disassemble all the valve and driveshaft parts from the Teflon block before steam sterilization**. The high temperature may cause the machined holes in the Teflon to become deformed by the parts pressed into them. Reinstall the valves and driveshaft after the Teflon cools down.

The Kynar luer fittings have been installed using silicone adhesive to hold them in place and prevent leaks. It should not be necessary to remove these fittings from the Teflon because of the sterilization process.

Can washing be done with [reagent]?

These materials of construction will withstand virtually all biological reagents and cold sterilization agents (such as Cidex, Cidex OPA, Mucasol, etc).

Parts List

Part		Part #
Controller Assembly		30280
Drive Motor Assembly		31206
Arbor (with O-Ring)		31204
Arbor Spacer 6.3 mm	(2 ea)	31056
Arbor Spacer 12.7 mm	(2 ea)	31057
Arbor Spacer 19 mm	(2 ea)	31058
Arbor 1.0 mm Cannula	(2 ea)	31051
Arbor 1.5 mm Cannula	(2 ea)	31052
Arbor 2.0 mm Cannula	(2 ea)	31053
Arbor 2.5 mm Cannula	(2 ea)	31054
Arbor 3.0 mm Cannula	(2 ea)	31055
Arbor Flat Tissue Holder		31186
Drive shaft Assembly		30203
Fittings, Luer	(10 ea)	31136
Function Control Valve Knob		30209
Level Control Valve Knob		30208
Reservoir Cover		30199
O-Ring	(10 ea)	31205
Reservoir Bottle 500 mL		31105
Reservoir Bottle 1,000 mL		31104
Screw Flat Head (level cap)		30288
Screw 10-24 x 3/8	(10 ea)	30283
Swabble Ports	(25 ea)	31081

Rev	Date	Changes
1.3	Nov 1, 2013	Update spacer lengths Update part numbers
1.4	Jan 1, 2014	Part Number descriptions updated and new picture for component ID

HARVARD APPARATUS
REGENERATIVE TECHNOLOGY

84 October Hill Road, Holliston MA 01746 USA
774.233.7300
www.HARTregen.com
cservice@HARTregen.com